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REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322 AND 1.323
Docket No. G-029US05DIV

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Bernard Bihain, Lydie Bougueleret, Frances Yen-Potin
Issued	:	November 6, 2007
Patent No.	:	7,291,709
For	:	LSR Receptor, Its Activity, Its Cloning, and Its Applications to the Diagnosis, Prevention and/or Treatment of Obesity and Related Risks or Complications

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 CFR 1.322 (OFFICE MISTAKE) and 1.323 (APPLICANTS' MISTAKE)

Sir:

A Certificate of Correction for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:	**Application Reads:**
Column 4, line 14:	Page 4, line 36:
"an a subunit"	--an α subunit--

Column 10, line 10:	Page 13, line 9:
"triglyceridernia"	--triglyceridemia--
Column 33, line 18:	Page 44, line 10:
"lad gene"	--lacl gene--
Column 38, line 11:	Page 50, line 19:
"triglyceridernia"	--trigylceridemia--
Patent Reads:	**Application Should Read:**
Column 40, line 56:	Page 54, line 2:
"portions thererof"	--portions thereof--
Patent Reads:	**Application Reads:**
Column 40, line 57:	Page 54, line 2:
"(Isr1.HS;"	--(lsr1.HS;--
Column 40, line 58:	Page 54, line 3:
"(Isr1.Rn;"	--(lsr1.Rn;--
Column 40, line 59:	Page 54, line 3:
"(Isr1.Mm;"	--(lsr1.Mm;--
Patent Reads:	**Application Should Read:**
Column 44, line 50:	Page 59, line 14:
"$-D-thiogalactopyranoside."	--β-D-thiogalactopyranoside.--

Patent Reads:	**Application Reads:**
Column 44, lines 52-53:	Page 59, line 15:
"deoxyribonuclease 1"	--deoxyribonuclease I--
Column 45, line 26:	Page 60, line 7:
"*E. coli* D115"	--*E. coli* DII5--
Patent Reads:	**Application Should Read:**
Column 45, line 58:	Page 60, line 26:
"proteincomplex"	--protein complex--
Patent Reads:	**Application Reads:**
Column 47, line 42:	Page 63, line 7:
"5% (WN)"	--5% (W/V)--
Column 55, line 65:	Page 74, line 27:
"1-lipoproteins"	--^{125}I-lipoproteins--
Column 56, line 63:	Page 76, line 2:
"triglyceridernia"	--triglyceridemia--
Column 60, lines 6-7:	Page 80, line 6:
"three α, α and β subunits"	--three α, α' and β subunits--
Column 64, line 15:	Page 85, lines 17-18:
"the α or α and β subunits"	--the α or α' and β subunits--

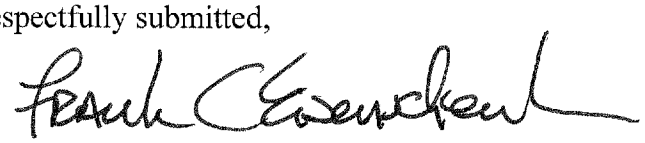
Column 66, line 62:	Page 89, line 1:
"Mads n, P.,"	--Madsen, P.,--
Column 66, line 64:	Page 89, line 3:
"Rail, S.C.,"	--Rall, S.C.,--
Column 67, line 17:	Page 89, line 19:
"*Proc. Natl. Aced. Sci.*"	--*Proc. Natl. Acad. Sci.*--
Column 67, line 52:	Page 90, line 8:
"Karisson, L.,"	--Karlsson, L.,--
Column 68, line 3:	Page 90, line 13:
"Strafford-Perricaudet, L."	--Stratford-Perricaudet, L.--
Column 68, line 12:	Page 90, line 20:
"Wilinow, T. E.,"	--Willnow, T. E.,--
Column 68, line 45:	Page 91, line 8:
"Homick, C. A.,"	--Hornick, C. A.,--
Patent Reads:	**Application Should Read:**
Column 183, line 7:	Amendment dated May 24, 2007, claim 1, line 4:
"clathrin binding leptin binding"	--clathrin binding, leptin binding--.

True and correct copies of pages 4, 13, 44, 50, 54, 59, 63, 74, 76, 80, 85, 89, 90 and 91 of the Specification as filed which support Applicants' assertion of the errors on the part of the Patent Office accompany this Certificate of Correction.

The fee of $100.00 was paid at the time this Request was filed. The Commissioner is also authorized to charge any additional fees as required under 37 CFR 1.20(a) to Deposit Account No. 19-0065.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,



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Attachments: Certificate of Correction
Copies of pages 4, 13, 44, 50, 54, 59, 63, 74, 76, 80, 85, 89, 90 and 91 of the specification as filed

of particles high in triglycerides of intestinal origin (Mann et al., 1995). This activity, expressed most particularly at the hepatic level, is dependent on the presence of free fatty acids which, by binding to the receptor, induce a reversible change in the conformation of this complex and allow it to bind, with a high affinity, various classes of lipoproteins such as those containing apoprotein B or apoprotein E.

On the other hand, under normal conditions, in the absence of free fatty acids, the complex receptor LSR does not bind lipoproteins, but is capable of binding a cytokine, in particular leptin, and then of internalizing it and of degrading it.

The present invention therefore relates to a purified LSR receptor, in particular of hepatic cells, characterized in that it is capable, in the presence of free fatty acids, of binding lipoproteins, and in the absence of free fatty acids, of binding a cytokine, preferably leptin.

According to the invention, this LSR receptor is, in addition, characterized in that the bound lipoproteins or the bound cytokine are incorporated into the cell and then degraded, the bound lipoproteins containing in particular apoprotein B or E.

It should be understood that the invention does not relate to the LSR receptors in a natural form, that is to say that they are not taken in their natural environment but obtained by purification from natural sources, or alternatively obtained by genetic recombination, or alternatively by chemical synthesis and capable, in this case, of containing non-natural amino acids, as will be described below. The production of a recombinant LSR receptor, which may be carried out using one of the nucleotide sequences according to the invention, is particularly advantageous because it makes it possible to obtain an increased level of purity of the receptor.

More particularly, the invention relates to a purified rat LSR receptor, characterized in that it comprises at least one subunit having a molecular weight of about 66 kDa and a subunit having a molecular weight of about 58 kDa.

Preferably, the purified rat LSR receptor of the present invention is characterized in that it contains an α subunit comprising the amino acid sequence of SEQ ID 2 or a sequence homologous thereto, or an α' subunit comprising the amino acid sequence of SEQ ID 4 or a sequence homologous thereto, and one, preferably three, β subunits comprising the amino acid sequence of SEQ ID 6 or a sequence homologous thereto.

The invention also relates to a purified mouse LSR receptor, characterized in that it comprises at least one subunit having a molecular weight of about 66 kDa and a subunit having a molecular weight of about 58 kDa.

Preferably, the purified mouse LSR receptor of the present invention is characterized in that it contains an α subunit comprising the amino acid sequence of SEQ

obesity. It is indeed essential to reduce the concentrations of leptin in obese human subjects in order to restore the physiological fluctuations of this hormone.

Accordingly, it is possible to envisage using compounds for the treatment of obesity allowing modulation of the number of LSR receptors, of their recycling rate, or of the change in their conformation, and/or allowing in particular:

1. leptinemia, and therefore the sensations of satiety and of hunger, to be controlled;
2. normal leptin concentrations to be restored and normal regulation of dietary habit by the normal perception of the sensations of hunger and of satiety ;
3. triglyceridemia to be controlled;
4. the plasma concentrations of residues of chylomicrons, highly atherogenic particles, to be regulated.

The role played by the LSR receptor in the hepatic clearance of lipoproteins of intestinal region makes it possible to envisage using compounds capable of modulating the expression and/or the activity of LSR in order to modulate the distribution of lipids of dietary origin between the peripheral tissues, in particualr the adipose tissues, and the liver. A treatment of obesity will consist in promoting the hepatic degradation of lipoproteins, and thereby reducing their storage in the adipose tissue, and regulating their plasma concentrations. The latter effect makes it possible to envisage the use of such compounds to reduce the risks associated with obesity, in particular the atherogenic risks.

Treatments of anorexia and of cachexia

It is possible to envisage using methods of regulating the activities of LSR to introduce treatments which make it possibile to overcome the vicious circle which characterizes anorexia nervosa. By reducing the number of receptors, it should be possible to promote weight gain in anorexic or undernourished subjects.

Under these conditions, it is advantageous to selectively inhibit the clearance of leptin by using synthetic peptides or pharmacological molecules which either reduce the synthesis of LSR or block its capacity to bind leptin and/or lipoproteins, or alternatively increase the catabolism of the receptor.

Treatment of abnormalities in the metabolism of cytokines

Analysis of the primary structure of the α subunit of LSR, as described below, shows a site homologous to the cytokine binding sites present on their receptors, as well as two routing signals which allow endocytosis and rapid degradation of ligands in the lysozomes. This observation is new in the sense that the cytokine receptors do not allow

modulate the activity of the LSR receptor. For example, such molecules can be used to stimulate or reduce the degradation of lipoproteins, preferably of lipoproteins high in triglycerides, or of cytokines, preferably of leptin. Such molecules can also be used to inhibit the activation by leptin or the activation by free fatty acids of the LSR activity.

Numerous methods exist for identifying ligands for the LSR receptor. One of these methods is described in Patent US 5,270,170, whose teaching is incorporated by reference. Briefly, a library is constructed which consists of random peptides, comprising a plurality of vectors each encoding a fusion between a peptide which is a candidate for binding to the LSR receptor and a protein binding to DNA such as the Lac repressor encoded by the lacI gene. The vectors for the library of random peptides also contain binding sites for the proteins binding to DNA such as the LacO site when the protein is the Lac repressor. The library of random peptides is introduced into a host cell in which the fusion protein is expressed. The host cell is then lysed under conditions allowing the binding of the fusion protein to the sites of the vector.

The vectors which have bound the fusion protein are brought into contact with the immobilized LSR receptor, a subunit of the immobilized LSR receptor or a fragment of the immobilized LSR receptor under conditions allowing the peptides to bind specifically. For example, the LSR receptor, a subunit thereof or a fragment thereof comprising at least 10, at least 20, at least 30, or more than 30 consecutive amino acids can be immobilized by binding to a surface such as a plate or a plastic particle.

The vectors which encode the peptides capable of binding to the LSR receptor are specifically retained at the surface by interactions between the peptide and the LSR receptor, a subunit of the receptor or a fragment thereof.

Alternatively, molecules capable of interacting with the LSR receptor can be identified using a double hybrid system such as the Matchmaker Two Hybrid System 2. According to the instructions of the manual accompanying the Matchmaker Two Hybrid System 2 (Catalogue No. K1604-1, Clontech), whose teaching is incorporated by reference, the nucleic acids encoding the LSR receptor, a subunit thereof or a fragment thereof comprising at least 10, at least 20, at least 30, or more than 30 consecutive amino acids are inserted into an expression vector so that they are in phase with the DNA encoding the DNA binding domain of the transcription activator of yeast GAL4. The nucleic acids of a library encoding proteins or peptides capable of interacting with the LSR receptor are inserted into a second expression vector so that they are in phase with the DNA encoding the activation domain of the GAL4 activator. The yeasts are transformed with the two expression plasmids and they are placed in a medium which makes it possible to select the cells expressing markers contained in each of the vectors as well as those expressing the HIS3 gene whose expression is dependent on GAL4. The

the invention, and/or a specific increase in the clearance activity for cytokines, in particular leptin, of the said receptor, and/or a specific increase in the clearance activity for lipoproteins, of the said receptor.

Also preferred are the compounds characterized in that they allow a decrease in the total activity or in the expression of the receptor according to the invention, and/or a specific decrease in the clearance activity for cytokines, in particular leptin, of the said receptor, and/or a specific decrease in the clearance activity for lipoproteins, of the said receptor.

Also preferred are the compounds characterized in that they allow modulation of the elimination of the cytokines, in particular leptin, and/or modulation of the elimination of the lipoproteins, chylomicron residues, and/or triglycerides.

The invention also comprises the compounds according to the invention, characterized in that they allow modulation of the level of cytokines, in particular leptinemia, and/or modulation of the level of lipoproteins, chylomicron residues, and/or triglycerides.

The compounds according to the invention, characterized in that they allow control of the level of cytokines, in particular leptinemia, are more particularly preferred.

Still preferably, the invention comprises the compounds according to the invention, characterized in that they allow control, preferably a decrease, of the level of lipoproteins, a decrease in the plasma concentration of chylomicron residues, and/or a decrease in triglyceridemia.

Among the compounds which are most preferred, there are preferred those characterized in that they are chosen from:

a. an antibody according to the invention;
b. a polypeptide according to the invention;
c. a polypeptide according to the invention, characterized in that it corresponds to a soluble form of the receptor according to the invention;
d. a vector according to the invention;
e. a vector according to the invention, characterized in that it has on its outer surface a site for specific recognition of hepatic cells;
f. a vector according to the invention, characterized in that the product of expression of the nucleic acid inserted by the vector into the target cell is either anchored in or excreted by the said transformed target cell;
g. a sense or antisense oligonucleotide according to the invention;
h. a leptin, or one of its protein variants, or a leptin which is chemically modified or which is modified by genetic recombination, or one of their fragments.

The invention finally relates to the compounds according to the invention as a medicament.

FIGURE 7: Alignment of the nucleotide sequences of the long forms of cDNA (encoding the α subunit) or portions thererof for human LSR (lsr1.HS; nucleotides 1 to 2062 of SEQ ID NO:7), rat LSR (lsr1.Rn; SEQ ID NO:1) and mouse LSR (lsr1.Mm; SEQ ID NO:13). The nucleotides conserved in the three sequences are identified by an * sign placed under the sequences. Dashes are added inside the sequences when the optimum alignment of the sequences cannot be achieved without creating microdeletions.

A : Alignment shown from amino acid positions 1 to 486 of SEQ ID NO:1.

B : Alignment shown from amino acid positions 487 to 1026 of SEQ ID NO:1.

C : Alignment shown from amino acid positions 1027 to 1551 of SEQ ID NO:1.

D : Alignment shown from amino acid positions 1552 to 2080 of SEQ ID NO:1.

E : Alignment shown from amino acid positions 2081 to 2097 of SEQ ID NO:1.

FIGURE 8 : Identification of the LSR receptor by ligand and Western blotting on solubilized proteins of rat liver membranes (lanes 1, 2 and 4), or on the partially purified protein of 240 kD (lane 3).

Lanes 1, 2 and 3 : Ligand blotting. Lane 1 : in the absence of oleate and of ^{125}I-LDL; lane 2 : in the presence of oleate and of ^{125}I-LDL; lane 3 : in the presence of oleate and of ^{125}I-LDL.

Lane 4 : Western blotting with anti-LSR antibodies.

FIGURE 9 : Effect of anti-LSR antibodies on the LSR activity.

A. Binding of ^{125}I-LDL onto the plasma membranes of rat hepatocytes in the presence of oleate and of increasing concentrations of anti-LSR antibody (v) or of control antibody (o), expressed as % of the total quantity of ^{125}I-LDL bound in the absence of antibodies.

B. Binding, incorporation and degradation of ^{125}I-LDL in rat hepatocytes in primary culture in the presence of oleate and of anti-LSR antibody (v) or of control antibody (o), expressed respectively as % of the binding, incorporation and total degradation of ^{125}I-LDL in the presence of non-specific antibodies.

FIGURE 10 : Identification of the LSR receptor by immunoprecipitation of ^{35}S-methionine- and ^{35}S-cysteine-labelled hepatocyte lysates, in the presence of control antibodies (lane 1), or of anti-LSR antibodies (lanes 2 to 4), after separation by electrophoresis under nonreducing (lanes 2 and 3) or reducing (lanes 1 and 3) conditions.

FIGURE 11 : Cloning of the cDNA encoding α and β-LSR.

A. Northern-blot analysis showing several sizes of LSR messenger RNA.

B. Multi-tissue Northern-blot analysis of LSR mRNA with a probe specific for LSR and a control probe specific for β-actin.

Preparation and radiolabelling of the lipoproteins

The VLDLs (d < 1.006 g/ml) and LDLs (1.025 < d < 1.055 g/ml) are isolated by sequential ultracentrifugation of fresh plasma from volunteers (Bihain and Yen, 1992; Goldstein et al., 1983) and used before 2 weeks. The lipoproteins are radioiodinated (Bilheimer et al., 1972) and used less than one week after the labelling. ^{125}I-LDL and ^{125}I-VLDL are filtered (0.22 μm membrane, Gelman) immediately before use.

Preparation and radiolabelling of mouse recombinant leptin

The leptin cDNA is obtained from the mRNA of adipose tissue of the mouse C57BL/6J by PCR. The 5' PCR primer introduces an initiation codon after the signal sequence which is deleted and a sequence encoding a hexahistidine end. The modified sequence encoding murine leptin is cloned into an expression vector pSE280 (Invitrogen, France) and expressed in *E. coli* The sequencing of the plasmid DNA confirms the coding sequence. The bacteria are cultured at 37°C and the synthesis of the protein is induced by 1 mM isopropyl $-D-thiogalactopyranoside. The bacteria, recovered after gentle centrifugation, are lysed by freeze-thaw and the DNA is digested with a deoxyribonuclease I. The cellular membranes are extracted with the aid of a detergent and the inclusion bodies are separated after centrifugation. After 3 washes in 1% sodium deoxycholate in PBS, the inclusion bodies are solubilized in a 6 M guanidine HCl solution. The renaturation of the recombinant protein is achieved by diluting 1/100 in PBS. The renatured protein is then purified and concentrated on a nickel-based chelate metal affinity chromatography column (Probond, Invitrogen). The elution is carried out with imidazole. The purity of the recombinant leptin is controlled by SDS-PAGE electrophoresis and its activity by the evaluation of satiety in mice C57BL/6J ob/ob after intraperitoneal injection of 25 μg of leptin. The recombinant leptin is then radiolabelled using Iodobeads (Pierce) and according to the method recommended by the manufacturer.

Cloning of the AdipoQ mRNA. Production and purification of recombinant AdipoQ proteins

Cloning of the cDNA into an expression vector

Mouse adipose tissue is obtained from C57Bl/6J mice and the mRNA is extracted with the aid of polydTs bound to magnetic beads (Dynabeads, Dynal, France). A cDNA library is constructed from mouse adipose tissue by reverse transcription at 40°C using a commercial kit (Superscript Life Technologies) using the supplier's instructions. The cDNA specific for AdipoQ is amplified using the following two primers :

5' CTACATGGATCCAGTCATGCCGAAGAT 3' (SEQ ID 37)

5' CGACAACTCGAGTCAGTTGGTATCATGG 3' (SEQ ID 38).

unbound oleate is then removed by 6 washes. The pellets are resuspended in 250 µl of incubation buffer, sonicated for 5 seconds, power 1.90% in the active cycle, and then centrifuged for 15 min at 18,000 rpm. The activated membranes are incubated for 1 hour at 4°C with various concentrations of antibody and then with 5 µg/ml of ^{125}I-LDL (1 hour at 4°C). 25 µl of 2% BSA are added to the incubation mixture. The quantity of ^{125}I-LDL bound to the membranes is measured by sedimenting the membranes by centrifugation after having deposited 200 µl of the incubation mixture on a layer of 5% (W/V) of BSA in buffer A. The supernatants are removed by aspiration, the tube bottoms are cut off and their radioactivity is counted in a γ counter.

The inhibitory effect of anti-LSR antibodies on the LSR activity, compared to that of any preparation of rabbit immunoglobulins is shown in Figure 9 A. The inhibition of the LSR activity by the anti-LSR antibodies confirms that the multimeric complex described above is responsible for the activity of the receptor and validates the ligand blotting technique used to identify it. The effect of the anti-band A antibodies was, in addition, tested on the other steps of the activity of the receptor : the internalization and the degradation of lipoproteins by the LSR expressed at the surface of hepatocytes in primary cultures.

Measurement of the binding, internalization and degradation of lipoproteins by hepatocytes

The LSR activity in the primary cultures of rat hepatocytes is measured by the binding, internalization and degradation of ^{125}I-LDL and ^{125}I-VLDL (LDL : low-density lipoprotein ; VLDL : very low-density lipoprotein), as described in Bihain and Yen, 1992 and Mann et al., 1995.

To measure the effect of the anti-LSR antibodies on the binding, internalization and degradation of LDLs by LSR, primary cultures of rat hepatocytes (48 h after plating) are incubated in the presence of 20 ng of leptin/well for 30 min at 37°C, followed by the addition of anti-LSR antibodies in the presence or in the absence of oleate. After incubating at room temperature for 30 min, ^{125}I-LDL (20 µg/ml) is added and then the cells are incubated for 4 h at 37°C. The binding, incorporation and degradation of ^{125}I-LDL are measured as described in Bihain and Yen, 1992 and Mann, et al., 1995.

The data in Figure 9 B show that the anti-band A antibodies inhibit most of the activity of binding of the LDLs to the LSRs present at the level of the hepatocytes. This inhibition induces a decrease in the same proportions in the internalization and proteolytic degradataion of the lipoproteins.

As shown in Figure 22, the hepatic capture of leptin is reduced and the renal capture is increased by the anti-LSR antibodies, compared with the control immunoglobulins.

These results therefore indicate that LSR is responsible for the selective hepatic capture of leptin and that a reduction in the number of receptors is observed in the obese animals. Such a reduction may explain the leptin-resistance syndrome and the increase in the plasma concentration of leptin which is observed in most obese human subjects.

It is also possible that the LSR receptor serves as degradation pathway for other cytokines, in particular those produced by the adipose tissue. The importance of Tumor Necrosis Factor α and Nerve Growth Factor will be noted in particular. These two cytokines exert a significant slimming effect when they are injected into human subjects (Cytokines and their receptors, 1996).

Example 5 : Control of the LSR activity by cytokines

The α subunit of the LSR receptor binds leptin and possesses potential phosphorylation sites. This makes it a receptor which not only mediates endocytosis, but could also serve in cell signalling.

The inventors therefore tested the hypothesis according to which leptin modulates the activity of LSR, as described below.

Measurement of the LSR activity of binding, internalization and degradation of lipoproteins in the presence of leptin

Rat hepatocytes in primary culture are incubated at 37°C for 30 min with an increasing concentration of leptin, and then incubated at 37°C for 4 hours with either 50 µg/ml of ^{125}I-LDL (specific activity : 209 cpm/ng) or 50 µg/ml of ^{125}I-VLDL (specific activity : 157 cpm/ng) in the absence or in the presence of 500 µM oleate. The cells are then washed and the quantities of ^{125}I-lipoproteins bound, incorporated and degraded are measured as described above in Example 1 (Bihain and Yen, 1992). The results shown in Figure 23 represent the differences obtained between the cells incubated with or without oleate. Each point represents the mean of 3 measurements. The standard deviation for each point is included in the symbol.

The addition of increasing concentrations of leptin to hepatocytes in culture increases the binding, internalization and degradation of VLDLs and LDLs (Figure 23).

disodium EDTA, and after separating the plasma by centrifugation, the plasma concentration of triglyceridemia is determined with the aid of an enzymatic assay kit. Each point on the curves presented corresponds to the mean with standard deviation obtained for 3 measurements per animal and for 3 different animals.

Measurement of the effect of leptin on the postprandial lipemic response in mice

The procedure is the same as above, except that immediately after the meal (time = 0 hour), the mice are injected intravenously with either 200 µl of physiological saline solution, or 200 µl of the same solution containing 50 µg of murine recombinant leptin.

Measurement of the postprandial lipemic response in mice in the presence of lactoferrin and/or leptin

ob/ob mice, starved since the day before, are force-fed with a meal identical to that described above. Immediately after the meal (time = 0 hour), the mice are injected intravenously with 200 µl of saline solution containing either no supplement, or 0.5 µg of leptin, or 2.5 mg of lactoferrin or alternatively a mixture of 0.5 µg of leptin and 2.5 mg of lactoferrin. Blood is collected between 2 and 3 hours after the meal and the plasma concentration of triglycerides (TG) is measured. The values obtained represent the mean with standard deviation obtained for 4 measurements per animal and for 2 different animals [$p < 0.02$ (*ob/ob* compared with *ob/ob* + leptin), $p < 0.01$ (*ob/ob* compared with *ob/ob* + lactoferrin), NS (*ob/ob* + lactoferrin compared with *ob/ob* + leptin + lactoferrin)].

In agreement with the reduction in the number of LSR receptors observed in the obese mice, an amplification of the postprandial lipemic response also exists in the untreated obese mice. The administration of leptin by the intravenous route, at the same time as the test meal, makes it possible to reduce the postprandial lipemic response in the two obese mouse lines and in the control mice (Figure 25).

This reduction in the lipemic response induced by leptin is suppressed by the administration of lactoferrin (Figure 26), which blocks the activity of LSR (Yen et al., 1994; Mann et al., 1995). This strongly suggests that the reduction in the lipemic response is explained by an increase in the LSR activity.

Finally, also *in vivo*, the administration of leptin induces an increase in the apparent number of LSR receptors expressed at the level of the surface of the hepatocytes. This increase is significant both in the ob/ob mice and in the db/db mice (Figure 27).

downstream at position 83 of the protein sequence. Furthermore, it is quite possible that this initiation codon is more upstream in the 5' region of exon 1 not yet determined or in a possible exon preceding the latter.

Finally, Figures 3A and 3B represents a schematic representation of the various protein forms identified in humans, indicating the conserved motifs.

This analysis makes it possible to conclude that three α, α' and β subunits of LSR, which are equivalent to the LSR 66, LSR 64 and LSR 58 forms in rats, exists in humans.

Identification and isolation of the genomic sequence for human LSR

Screening of public data banks of nucleic sequences (Genebank, version: 101) both with the sequence of mouse lisch7 (Accession No.: U49507) and with that of rat LSR_2097 isolated by the inventors made it possible to isolate two human genomic DNA sequences. They are cosmids whose accession numbers are AC002128 and AD000684, of respective sizes 45,328 bp and 41,936 bp. These two cosmids partially overlap. The 3' end of the cosmid AC002128 overlaps, over 12838 bp, the 5' end of the cosmid AD000684. On the common portion of 12,838 bp, the sequences are 100% identical, apart from two deletions at positions 822 and 3170 of the cosmid AD000684. The human LSR gene is distributed over the two cosmids. To facilitate the study of this region, a complete genomic sequence was reconstituted: the 45,328 bp of the cosmid AC002128 were added to the sequence of the cosmid AD000684 between the 12,839 base and the 41,936 base. The combination constitutes a sequence of 74,426 bp. A genomic sequence covering the LSR gene, was extracted (SEQ ID 19).

The putative exons of the LSR gene were determined after alignment of the sequence described above with the sequences of the RNAs for mouse Lisch7 and rat LSR. The validity of the splicing sites on either side of the putative exons was verified.

Moreover, a human genomic library consisting of BACs was screened by the methods described in Chumakov et al., 1995 ; the clones thus isolated were contiged, subcloned and then sequenced in order to obtain the human genomic sequence encoding LSR (SEQ ID 41).

The two sequences thus obtained (SEQ ID 19 and 41) carry minor differences which are mentioned in the accompanying listings.

Several proteins can interact with gC1q-R because they exhibit homologies with complement C1q. In particular, two proteins isolated in mice, AdipoQ (Hu et al., 1996) and acrp30 (Scherer et al., 1995), and a human protein APM1 (Maeda et al., 1996) exhibit marked homologies. These three proteins, like the components of complement C1q (C1q A, B, C), are secreted proteins; they have an NH_2-terminal end which resembles collagen (repetition of Gly-X-Y motifs) and a COOH-terminal end corresponding to the globular domain of complement Clq. These three proteins are preferably expressed in the adipose tissue. There are only 3 amino acids differing between AdipoQ and acrp30. APM1, a protein whose messenger has been characterized as being highly expressed in adipocytes, exhibits 79.7% nucleic acid identity and 80.6% amino acid identity with AdipoQ. APM1 is therefore certainly the human homologue of AdipoQ.

Example 13 : Screening of compounds modifying the activity of the LSR receptor

As described above, the inventors formulated the hypothesis that the LSR " γ band ", a protein which is highly homologous to gC1qR, might interact with the LSR receptor like a molecular chaperone and might thus form an " LSR complex ", comprising the α or α' and β subunits of the LSR receptor and a gC1qR type molecule. gC1qR has been previously identified as a cell surface protein which binds the globular heads of the complement factor C1q. In addition to C1q, several proteins exhibiting homologies with the C1q proteins, in particular AdipoQ and acrp30 in mice and APM1 in humans, are capable of interacting with the protein homologous to gC1qR in the LSR complex and of modifying the LSR activity.

Screening parameters

The screening of a compound such as C1q or AdipoQ was carried out through the measurement of various parameters of which the most important is the measurement of the effect of the compound on the activity of the LSR receptor. The various parameters are the following :

- change in weight
- food intake
- postprandial lipemic response
- binding, internalization and/or degradation of lipoproteins such as the LDLs.

Change in weight

Osmotic pumps were surgically inserted into the abdominal cavities of 12 Sprague-Dawley male rats of 400-450 g. The osmotic pumps contained either 2 ml of PBS (phosphate buffered saline), pH 7.4 (control 6 rats), or 2 ml of recombinant AdipoQ

Honoré, B., Madsen, P., Rasmussen, H.H., Vandekerckhove, J., Celis, J.E., and Leffers, H. *Gene* **134** : 283-287, 1993.

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,291,709

APPLICATION NO.: 10/650,507

DATED : November 6, 2007

INVENTOR : Bernard Bihain, Lydie Bougueleret, Frances Yen-Potin

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 4,
Line 14, "an a subunit" should read --an α subunit--.

Column 10,
Line 10, "triglyceridernia" should read --triglyceridemia--.

Column 33,
Line 18, "lad gene" should read --lacl gene--.

Column 38,
Line 11, "triglyceridernia" should read --triglyceridemia--.

Column 40,
Line 56, "portions thererof" should read --portions thereof--.
Line 57, "(Isr1.HS" should read --(lsr1.HS--.
Line 58, "(Isr1.Rn" should read --(lsr1.Rn;--.
Line 59, "(Isr1.Mm;" should read --(lsr1.Mm;--.

Column 44,
Line 50, "$-D-thiogalactopyranoside." should read --β-D-thiogalactopyranoside--.
Lines 52-53, "deoxyribonuclease 1" should read --deoxyribonuclease I--.

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DATED : November 6, 2007

INVENTOR : Bernard Bihain, Lydie Bougueleret, Frances Yen-Potin

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 45,
Line 26, "*E. coli* D115" should read --*E. coli* DII5--.
Line 58, "proteincomplex" should read --protein complex--.

Column 47,
Line 42, "5% (WN)" should read --5% (W/V)--.

Column 55,
Line 65, "1-lipoproteins" should read --^{125}I-lipoproteins--.

Column 56,
Line 63, "triglyceridernia" should read --trigylceridemia--.

Column 60,
Lines 6-7, "three α, α and β subunits" should read --three α, α' and β subunits--.

Column 64,
Line 15, "the α or α and β subunits" should read --the α or α' and β subunits --.

Column 66,
Line 62, "Mads n, P.," should read --Madsen, P.,--.

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It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 66,
Line 64, "Rail, S.C.," should read --Rall, S.C.,--.

Column 67,
Line 17, "*Proc. Natl. Aced. Sci.*" should read --*Proc. Natl. Acad. Sci.*--.
Line 52, "Karisson, L.," should read --Karlsson, L.,--.

Column 68,
Line 3, "Strafford-Perricaudet, L." should read --Stratford-Perricaudet, L.--.
Line 12, "Wilinow, T. E.," should read --Willnow, T. E.,--.
Line 45, "Homick, C. A.," should read --Hornick, C. A.,--.

Column 183,
Line 7, "clathrin binding leptin binding" should read --clathrin binding, leptin binding--.

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